基础研究

MiR-200b 在高糖环境下对视网膜血管内皮细胞功能的影响及机制

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摘要:目的 探讨高糖环境下MiR-200b对人视网膜血管内皮细胞(hRECs)的作用机制。**方法** 在高糖或正常环境培养hRECs,实时荧光定量PCR检测MiR-200b表达。MiR-200b转染hRECs,并且用MTT法检测高糖环境下MiR-200b对hRECs增殖的影响。**结果** 与正常对照组比较,高糖组VEGF和TGF-β1的mRNA和蛋白表达明显增加(P<0.05)。转染MiR-200b后,hRECs中MiR-200b表达升高,而VEGF和TGF-β1 mRNA和蛋白的表达显著降低。与高糖组相比,转染MiR-200b后hRECs增殖受到明显抑制(P<0.05)。**结论** MiR-200b可能通过改变VEGF和TGF-β1的表达,调节REC细胞生长和增殖,从而参与糖尿病视网膜病变发生、发展。

关键词:糖尿病视网膜病变;视网膜血管内皮细胞;血管内皮生长因子;转化生长因子β

Effect of MiR-200b on retinal endothelial cell function in high-glucose condition and the mechanism

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Abstract: Objective To investigate the effect of MiR-200b on human retinal endothelial cells (hRECs) cultured in high glucose and explore the mechanism. **Methods** hRECs cultured in high glucose or in normal media were examined for MiR-200b mRNA expression using real-time PCR. The effect of MiR-200b transfection on hREC proliferation in high-glucose culture was evaluated with MTT assay, and real-time PCR and Western blotting were performed to determine vascular endothelial growth factor (VEGF) and transforming growth factor β1 (TGFβ1) expression in the transfected cells. **Results** The cells in high-glucose culture showed significantly decreased MiR-200b expression and active proliferation. Compared with those in normal control cells, VEGF and TGFβ1 mRNA and protein expressions increased markedly in cells cultured in high glucose (P<0.05). MiR-200b transfection of the cells caused significantly increased cellular expression of MiR-200b but decreased expression levels of VEGF and TGFβ1 mRNA and protein, and suppressed hREC proliferation in high glucose culture (P<0.05). **Conclusion** MiR-200b can regulate REC growth and proliferation by changing VEGF and TGFβ1 expressions and thus play a role in the pathogenesis and progression of diabetic retinopathy.

Keywords: diabetic retinopathy; retinal endothelial cell; vascular endothelial growth factor; transforming growth factor β1

糖尿病性视网膜病变是糖尿病的重要并发症之一,是导致成人失明的主要原因[1-2]。它严重影响了患者的身心健康,给社会带来沉重经济负担[3]。据世界卫生组织调查,目前全世界有多达3.6亿糖尿病患者,预计到2030年将达到10亿[4-5]。研究发现,糖尿病视网膜病变(DR)与高糖环境对视网膜微血管系统损害密切相关[6]。DR可以影响视网膜结构,导致其代谢和功能紊乱。视网膜微血管内皮细胞负责视网膜神经营养需求。视网膜微血管内皮细胞维持血-视网膜屏障稳定,并清除毒素和炎症因子,他们在保护视力方面发挥了关键作用[7-8]。微小RNA,也称为miRNA或小RNA,是一种有调节功能的短链非编码RNA,广泛存在在动植物中[9]。miRNA可降解为mRNA并且通过与靶基因完全或不完全配对,或通过抑制下游靶蛋白的表达来调节蛋白质翻译[10]。miRNA参与各种生理过程,包括细胞增殖,凋亡,信号转导,分化,代谢和激素分泌以及维持胚胎干细胞的潜能。它能调节人体的生长发育,使人体适应环境。研究表明miRNA与癌症发生有关,目前,有关miRNA的大量研究主要集中在其对肿瘤发生,发展,侵袭,转移和其它生物特征的作用等方面[10],而有关miRNA在糖尿病

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视网膜病变中的作用研究未见报道。MiR-200b是一种新发现的miRNA,研究发现糖尿病患者的MiR-200b表达明显降低,并与糖尿病的发展密切相关。因此,MiR-200b被认为参与糖尿病的发生和发展[11-12]。进一步的研究表明,MiR-200b可能参与了DR的发生和发展[13]。但MiR-200b作用于视网膜内皮细胞的具体的功能和机制仍不清楚。高血糖状态会导致视网膜血管内皮细胞(REC)的直接或间接的损伤,可导致视力障碍和DR的发生。同时,血管内皮生长因子(VEGF)和转化生长因子β1(TGF-β1)被证实参与DR病变的调节机制[14-15]。本研究目的是探讨高糖状态下MiR-200b对RECS的影响和调节TGF-β1、VEGF的机制,分析MiR-200b在DR中的作用。

1 材料和方法

1.1 试剂与仪器

hRECs购于美国Sciencell;青霉素、链霉素和EDTA为Hyclone产品;Enzyme-EDTA购于Sigma;DMSO和MTT购自Gibco,RNA提取试剂盒,反转录试剂盒,lipo2000购自Invitrogen;ECL试剂来自Amersham biociences;兔抗人VEGF和TGF-β1抗体、标记IgG二抗HRP购于美国Cell signaling;Western blot购于上海Beyotime;DNA放大器和PCR仪为美国ABI产品,其他常见的试剂购于上海生工。

1.2 方法

1.2.1 hRECs培养和分组 将hRECs接种在不含胎牛血清,含5.5 mmol/L葡萄糖的DMEM培养基中(含100 U/mL青霉素和100 μg/mL链霉素),第8代细胞数达到 1×10^6 cells/cm²,将它们随机分为3组。包括正常情况下细胞培养的正常对照组;高糖组:在高糖环境下诱导培养72 h后,hRECs在含33 mmol/L葡萄糖的微血管内皮细胞培养基增殖。MiR-200b组:在高糖环境下,将MiR-200b转染hRECs。

1.2.2 MiR-200b转染 MiR-200b由在上海的公司模拟合成。其序列为5'-UAAUACUGCCUGGUAAUGAUGAC-3',在高糖环境下,用转染液lipo2000将MiR-200b转染hRECs。hRECs在增殖期,接种于 3×10^6 cells/cm²的6孔培养板,并在5% CO_2,37 ℃二氧化碳培养箱中培养12 h后,将混有MiR-200b的lipo2000转染液添加到细胞培养6 h,然后更换培养基后继续培养。

1.2.3 Real-time PCR 用Trizol从hRECs中提取mRNA,应用实时荧光定量PCR检测目标基因的表达情况。引物见表1。聚合酶链反应步骤:先为52 ℃,1 min,然后35个循环,包括90 ℃ 30 s,58 ℃ 50 s,72 ℃ 35 s。

1.2.4 MTT 细胞接种于96孔板后,取浓度为5 g/L的MTT溶液20 μL添加到每个孔中,孵育4 h。除去上清液后,加入150 μL DMSO,10 min后,采用紫外分光光度仪测570 nm处吸光度值,计算细胞增殖率。

表1 RT-PCR引物

Tab.1 Primer sequence used in RT-PCR

Gene	Forward 5'-3'	Reverse 5'-3'
GADPH	AGTGCCAGCCTCGTCTCATAG	CGTTGAACTTGCCGTGGGTAG
MiR-200b	AGCGGCTCATCTAAACAATGG	GGCGCACATTCTCTCCGTA
VEGF	AAACTGTCAGCTCGGTCAGA	TCAGGGGCCGATTAAAGCTC
TGFβ1	GCCAGGATATGAGTTTGGGA	GGGTGCATGTCTGCTCCTGT

1.2.5 Western blot 从超声处理的提取液中提取hRECs总蛋白。经过10 000 r/min离心15 min,取上清液转移到新的EP管,存放在−20 ℃冰箱。蛋白质由10%的聚丙烯酰胺凝胶电泳分离并转移到聚偏二氟乙烯膜(PVDF)膜,用5%脱脂奶封闭2 h后,与VEGF抗体(1∶1000)或TGF-β1抗体(1∶2000)在4 ℃共同孵育一整夜;用PBST漂洗后,膜被进一步与羊抗兔二抗体(1∶2000)一起孵育30 min,然后用化学发光剂显像。IPP6.0软件分析Western blot条带。

1.3 统计分析

用SPSS16.0对所有数据进行统计分析。数据用均数±标准差表示。多组间的差异进行单因素方差分析。$P<0.05$被认为有显著差异。

2 结果

2.1 hRECs中MiR-200b表达

实时荧光定量PCR检测hRECs中MiR-200b表达。如图1所示,在正常环境下,hRECs中MiR-200b高度表达,而高糖环境下则明显降低($P<0.05$)。转染MiR-200b的hRECs在高糖环境下MiR-200b水平显著增高($P<0.05$)。

2.2 MiR-200b对hRECs细胞增殖的影响

MTT法检测在高血糖环境下MiR-200b对hRECs细胞增殖的影响。结果发现,高糖环境促进hRECs明显增殖($P<0.05$)。转染MiR-200b的hRECs在高糖环

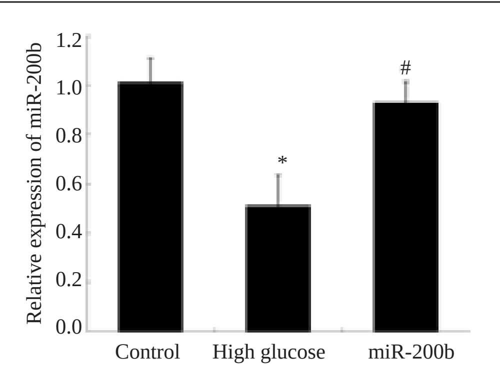


图1 MiR-200b在正常对照组,高血糖组和转染组的表达

Fig.1 MiR-200b expression in hRECs. *P<0.05 *vs* normal control; #P<0.05 *vs* high glucose group.

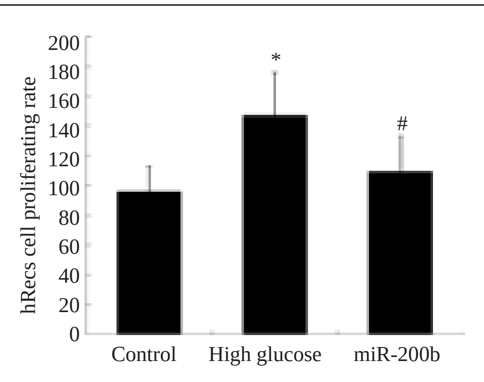


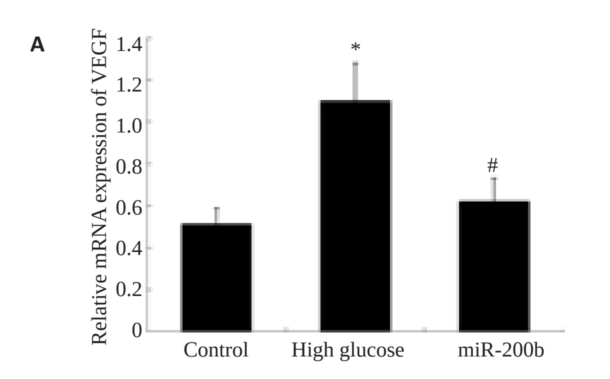
图2 MiR-200b对正常对照组,高血糖组和转染组hRECs增殖的比较

Fig.2 Effect of MiR-200b on hRECs proliferation. *P<0.05 *vs* normal control; #P<0.05 *vs* high glucose group.

境下细胞增殖受到明显抑制,这表明MiR-200b降低可以促进血管内皮细胞的显著增殖,而其增高能抑制靶细胞视网膜血管内皮细胞增殖(P<0.05,图2)。

2.3 MiR-200b对hRECs内VEGF和TGF-β1mRNA表达的影响

采用实时定量PCR检测在高血糖环境下MiR-200b对hRECs中VEGF和TGF-β1 mRNA的表达影响。高糖环境下VEGF和TGF-β1 mRNA在hRECs的表达明显升高(P<0.05)。高糖环境下转染MiR-200b的hRECs,VEGF和TGF-β1mRNA的表达明显受到抑制(P<0.05,图3)。



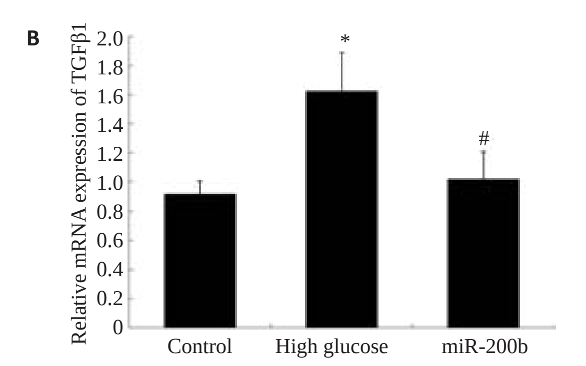


图3 MiR-200b对正常对照组,高血糖组和转染组VEGF和TGFβ1影响的比较

Fig.3 Effect of MiR-200b on VEGF (*A*) and TGFβ1 (*B*) mRNA expression in hRECs. *P<0.05 *vs* normal control; #P<0.05 *vs* high glucose group.

2.4 MiR-200b对hRECs中VEGF和TGF-β1蛋白表达的影响

用Western blotting测定hRECs转染MiR-200b后对VEGF和TGF-β1蛋白及mRNA的表达的影响。在高糖环境下hRECs中VEGF和TGF-β1蛋白表达水平明显升高(P<0.05);转染MiR-200b后VEGF和TGF-β1蛋白表达的水平受到明显抑制(P<0.05,图4、5)。

这表明,高糖环境可以抑制hRECs中MiR-200b,从而促进VEGF和TGF-β1 mRNA以及蛋白的表达,进一步引起视网膜血管内皮细胞结构和功能紊乱。转染MiR-200b后,靶向细胞促进其表达,并下调VEGF和TGF-β1 mRNA及蛋白的表达,从而改善糖尿病视网膜病变。

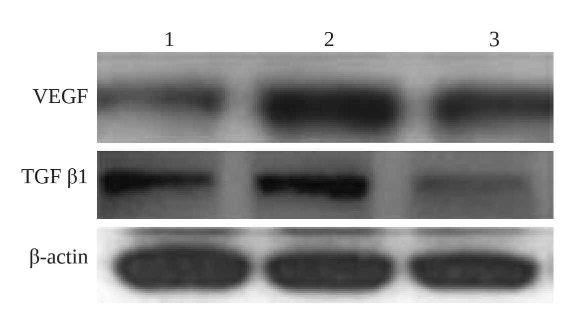


图4 MiR-200b对正常对照组,高血糖组和转染组hRECs中VEGF和TGFβ1蛋白表达的影响

Fig.4 Effect of MiR-200b on VEGF and TGFβ1 protein expression in hRECs. 1: Control; 2: High glucose group; 3: MiR-200b group.

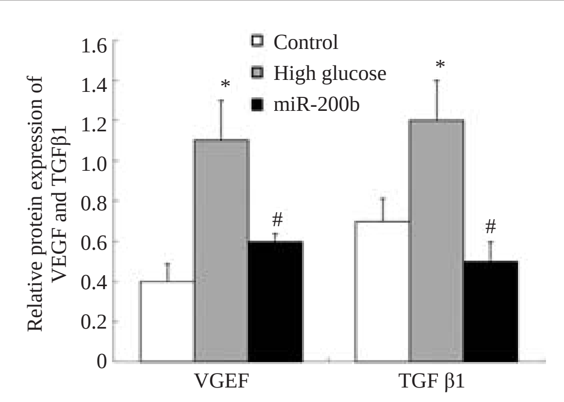


图5 MiR-200b对正常对照组,高血糖组和转染组hRECs中VEGF和TGFβ1蛋白表达分析

Fig.5 Effect of MiR-200b on VEGF and TGFβ1 protein expression in hRECs. *P<0.05 *vs* normal control; #P<0.05 *vs* high glucose group.

3 讨论

高血糖是导致糖尿病并发症发生发展的重要因素,糖尿病视网膜病变是一种常见的糖尿病微血管并发症,高血糖环境下糖尿病患者视网膜血管内皮细胞发生一系列内分泌代谢变化,并引起器官结构和功能紊乱[16-17]。高血糖可导致视网膜血管内皮细胞功能障碍,包括通过调节内皮素和血管内皮生长因子促进细胞增殖和新生血管形成[18-19]。

miRNA参与各种生理过程,包括细胞增殖,凋亡,信号转导,分化,代谢和激素分泌以及维持胚胎干细胞的潜能。MiR-200b是一种新发现的miRNA,它参与调节糖尿病的发生和发展[10],但其在糖尿病视网膜病变中的作用尚未阐明。通过hRECs培养和高糖环境处理,我们的研究证实,在高糖状态下hRECs中MiR-200b水平下降。这进一步表明,细胞可以通过转染MiR-200b抑制hRECs异常增殖。

血管内皮生长因子(VEGF)有多种亚型,通过结合相应受体(VEGFR)发挥其作用。大部分VEGFR位于内皮细胞表面,与VEGF特异性结合可改变血管通透性,促进血管形成。因此,对于早期的糖尿病视网膜病变,主要是通过对血管内皮生长因子的调节来改变血管通透性,晚期则参与糖尿病视网膜新生血管形成[20-21]。TGFβ1是一种多肽生长因子,广泛分布于众多细胞中,具有多种生物学功能。它调节细胞生长,分化和迁移,调节细胞外基质的合成和分泌,并参与免疫调节,在糖尿病视网膜病变的形成过程中发挥了重要的作用。TGFβ1还是一种促细胞增殖的重要调控因子,它能上调整联蛋白的表达,促进细胞外基质分泌,调节细胞与基质之间的相互作用,趋化巨噬细胞促进新生血管形成和成纤维细胞生长。它通过抑制抗体生成和淋巴细胞增殖抑制CTL、NK细胞、LAK细胞的细胞毒作用,发挥免疫抑制作用[22-23]。然而,目前有关TGFβ1在糖尿病视网膜病变中作用的研究未见报道。因此,我们重点研究了MiR-200b对视网膜血管内皮细胞的影响。研究证实,高糖环境抑制MiR-200b的表达,促进VEGF和TGF-β1 mRNA及蛋白的表达,从而促进内皮细胞增殖和新生血管形成,导致视网膜病变。通过上调MiR-200b表达,可以减少VEGF、TGF-β1 mRNA和蛋白的表达,改善血管内皮细胞的结构和功能,延缓DR的进展。

综上所述, MiR-200b通过改变VEGF和TGFβ1表达,调节视网膜血管内皮细胞的生长和增殖,可以延缓DR的进展。

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